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Estimated Susceptibility to Asymptomatic Secondary Immune Response Against Measles in Late Convalescent and Vaccinated Persons

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Serological evidence indicates that measles virus (MV) could circulate in seropositive, fully protected populations. Among individuals fully protected against disease, those prone to asymptomatic secondary immune response are the most likely to support subclinical MV transmission. The serological characteristics of protected subjects who developed secondary immune response after reexposure to measles have been described recently [Huiss et al. (1997): Clinical and Experimental Immunology 109:416–420]. On the basis of these data, a threshold of susceptibility was defined to estimate frequencies of secondary immune response competence in different populations. Among measles, late convalescent adults (n = 277) and vaccinated high school children (n = 368), 3.2–3.9% and 22.2–33.2%, respectively, were considered susceptible to secondary immune response. A second vaccination did not seem to lower this incidence. Even when estimates of symptomatic secondary immune response (e.g., secondary vaccine failure) were taken into account, susceptibility to subclinical secondary immune response was still 5–8 times higher after vaccination than after natural infection. Although viral transmission between protected individuals has never been directly demonstrated, the data describe a population in which protected but infectious persons could potentially be of epidemiological importance. ***J. Med. Virol. 56:85–90, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: IgG; neutralization; hemagglutination inhibition; measles virus

INTRODUCTION

Systematic vaccination has dramatically reduced measles morbidity at least in the industrialized world. Circulation of the virus, however, continues even in highly vaccinated populations [Gustafson et al., 1987; Markowitz et al., 1989]. In the absence of seronegative individuals, several observations indicate that measles virus (MV) can circulate also among seropositive persons without signs of clinical measles [Pedersen et al., 1989]. After reexposure to measles, specific IgG-positive subjects may sometimes present with mild disease [Aaby et al., 1986; Edmonson et al., 1990] and protected individuals can develop asymtomatic secondary immune responses [Gustafson et al., 1987; Pedersen et al., 1989; Ozanne and d'Halewyn, 1992]. Subclinical secondary immune response to measles has been observed in immunized populations [Gustafson et al., 1987; Pedersen et al., 1989; Ozanne and d'Halewyn, 1992]. With vaccination rates increasing, it becomes important to understand whether asymptomatic individuals may play a role in viral replication and transmission. This phenomenon could be studied more directly if the susceptible population would be clearly defined.

We recently investigated secondary immune response in parents exposed to their children with acute measles [Muller et al., 1996; Huiss et al., 1997]. Asymptomatic secondary immune response occurred in all exposed parents whose titers were below defined thresholds. Above these thresholds, all persons were protected against secondary immune response. Using these thresholds, the frequency of susceptibles to secondary immune response among late convalescent and vaccinated individuals were estimated to define a high-risk population in which asymtomatic viral transmission could potentially be investigated.

MATERIALS AND METHODS

Late Convalescent Sera

Serum samples were collected in our institute from 277 consecutive outpatients above 25 years of age who

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underwent venepuncture for unrelated reasons between December 1995 and January 1996. Of these, 103 were males (25–79 year old, median 50.1) and 174 were females (25–91 year old, median 42.3). It can be assumed that the vast majority of the persons in this age bracket had measles during their childhood because they were born at a time (1905–1970) when MV immunity was mostly acquired by early natural infection. Routine vaccination against measles was introduced in Luxembourg in 1975.

Early Convalescent Sera

With the support of the Direction de la Santé of the Ministry of Health, Luxembourg, a hot-line for measles surveillance was installed in the Department of Immunology of the Laboratoire National de Santé (LNS). Between March and July 1996, an outbreak of measles occurred in the cantons of Wiltz and Clervaux in the Grand-Duchy of Luxembourg, affecting at least 84 patients, mostly primary school children. Single or paired sera were obtained. Seventy serum samples drawn 14–76 days after onset of rash were included as early convalescent sera.

Sera From Vaccinated Children

Sera were also collected in September and October 1996 (i.e., after the above outbreak) from 503 children from a coed public and an all-girl private high school (the Lycée R. Schuman and the Lycée Fieldgen) in Luxembourg City and a small-town (Wiltz) coed technical high school. Only children in the first year were enrolled and participation was 63%, 76%, and 86%, respectively. The median age was 12.7 (11.0–15.5). The immunization status was recorded from vaccination documents: 73.2% (80.3%, 80.5%, 59.7%, respectively, for the three schools) of all children were vaccinated at least once. Among these, 9.7% (20.0%, 10.3%, and 3.6%, respectively) were vaccinated twice, and 26.8% (19.7%, 19.5%, 40.3%, respectively) had no vaccination records and were excluded from the study.

When the first vaccination was given, the median age of the children was 19.6 months (3–155 months). Only 5.2% were below the age of 15 months, the recommended age for vaccination. The second vaccination was given at the median age of 11.6 years (1.6–15.5 years). Pluserix (49%) and Rimparix (15%) were mainly used for the first vaccination. In 15.4% of children, other vaccines were used and 20.6% were unknown; 51.1% of the vaccines were trivalent, 17.4% divalent, 9.1% monovalent, and 22.4% unknown.

All sera were aliquoted and frozen/thawed not more than twice before being tested. All sera were drawn after informed consent from the donors or their parents or guardians.

Serological Assays

Hemagglutination inhibition (HI) and neutralization (NT) titers were determined following standard techniques as described by Huiss et al. [1997]. Using the Second International Standard for antimeasles serum obtained from the National Institute for Biological Standards and Control (Hertfordshire, UK) [Forsey et al., 1991], the threshold for positivity of the NT assay was found to be 250 mIU/ml, which corresponds to reported protective titers (>200–>255 mIU/ml) [Garenne et al., 1993; Miller et al., 1995; Ratnam et al., 1995]. A total of 5,000 IU/ml correspond to a dilution of $1:2^8$–2^9. MV-specific IgG was measured with a commercial ELISA kit based on continuously MV-infected permanent simian kidney cells (Enzygnost, Behringwerke Marburg, Germany) following the manufacturer's instructions (serum dilution 1:231). All data are presented as net IgG, corresponding to the difference in $mO.D._{450}$ between MV-infected and uninfected control wells. According to the manufacturer, ΔA of <100 mO.D. (150 mIU/ml) is negative, and >200 mO.D. (325 mIU/ml) is positive. Sera with intermediate values are recommended for retesting, and if not defined are considered "equivocal." Background levels were 53–169 (mean 87) mO.D. Reference sera with HI, NT, and IgG titers corresponding to the thresholds (see Fig. 2) were obtained by mixing suitable sera. These sera were used for quality control in the different assays.

Serological Thresholds for Asymptomatic Secondary Immune Response

The following combined thresholds were used to identified persons who are prone to develop secondary immune response [Huiss et al., 1997]: specific IgG, <780 to 870 mO.D., corresponding to <2147 to 2559 mIU/ml (Enzygnost Elisa kit, Beringwerke); NT, $<1:2^{5.5}$ to $<1:2^{6.5}$; and HI, $<1:2^{6.5}$ to $<1:2^7$. The intervals given correspond to an undefined zone between the highest value (excluded) of an individual with secondary immune response and the lowest value (excluded) of an individual without secondary immune response.

RESULTS

Serology of the Different Cohorts

Figure 1 shows the relative distribution of HI and NT titers, and specific IgG for late convalescent donors and high school children stratified according to their vaccination status. Sera from early convalescent measles patients, drawn when the antibody response was fully developed in most patients (i.e., 14–54 days after the onset of rash), are shown for comparison.

Early convalescents have significantly higher MV titers (by HI, NT, and specific IgG) than late convalescents or vaccinees (Table I). Late convalescent persons had significantly higher NT and HI titers than individuals vaccinated once. After a second vaccination, HI titers, but not NT titers, became similar to those of late convalescents. No statistical difference was found between children vaccinated once or twice, although some individuals with the lower HI titers seem to have converted to higher HI titers. Within the cohorts of the once- or twice-vaccinated subjects, no serological differences between boys and girls or between different schools were detectable by Student *t*-test. Similarly, no

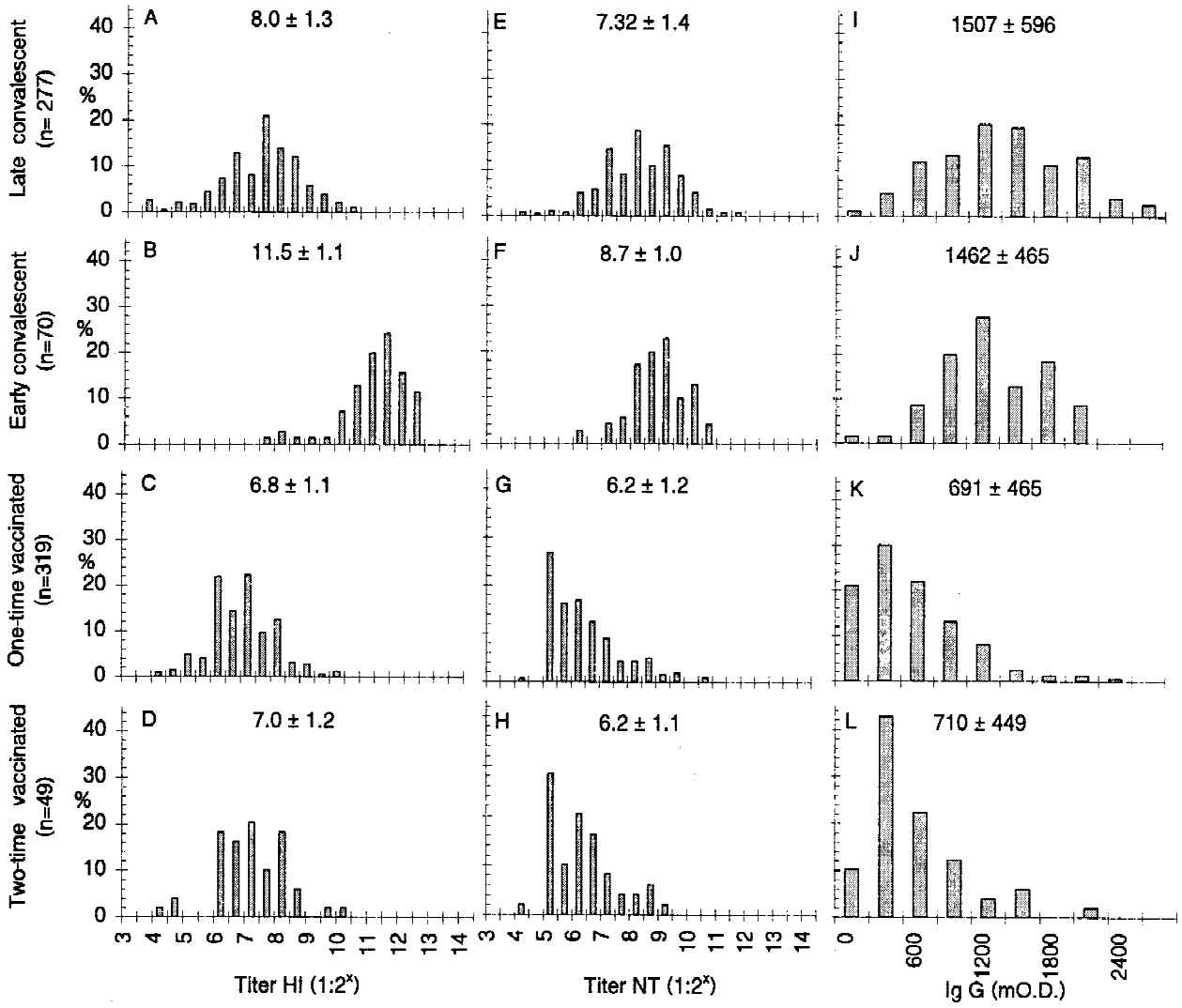

Fig. 1. Relative distribution of HI (panels **A–D)** and NT (panels **E–H)** titers ($-\log_2$ of serum dilutions) and specific IgG levels (net absorbance; Enzygnost; panel **I–L)** of late (A, E, I), early (B, F, J) convalescent donors and of children vaccinated once (C, G, K) or twice (D, H, L). Mean ± S.D. and size of cohort in parentheses are shown for each panel. Levels of significance (*P*) between values are shown in Table I. IgG levels are expressed in intervals of 300 mO.D. (0–300, 300–600 etc).

significant difference in titers was detected between the Rimparix and Pluserix group (data not shown).

Frequency of Secondary Immune Response Susceptibles

Figure 2 shows the HI and NT titers and levels of specific IgG of convalescent and vaccinated donors in relation to the thresholds for secondary immune response given in Materials and Methods [Huiss et al., 1997]. Different estimates of the frequency of secondary-immune-response-competent subjects are obtained, whether sera within the undefined zone were included or not (Table II). The more conservative (i.e., lower) estimate is obtained when individuals with values within the undefined region are considered secondary immune response resistant. As expected, none of the early convalescents fulfills the criteria of secondary immune response susceptibility (Fig. 2A); among the late convalescents, only 3.2–3.9% (1 male, 8 females; Fig. 2B) seem to be secondary immune response competent. However, among vaccinated high school children, the frequency of secondary immune response susceptibles was 7–8 times higher even when these were vaccinated twice (Fig. 2C and D). The estimated frequencies of secondary immune response susceptibility would be higher if only two parameters were considered, although a reasonable approximation would be obtained in a cohort of late convalescents but not in vaccinees by combining NT titers and Enzygnost data.

Late convalescents prone to secondary immune response tended to be younger in comparison with the average age of this cohort (33.6 versus 43.5 years; $P = 0.07$). All were born before 1973 (five before 1965); 63.5% were women.

Among the vaccinated children, no difference in sex distribution or in the type of vaccines used was found

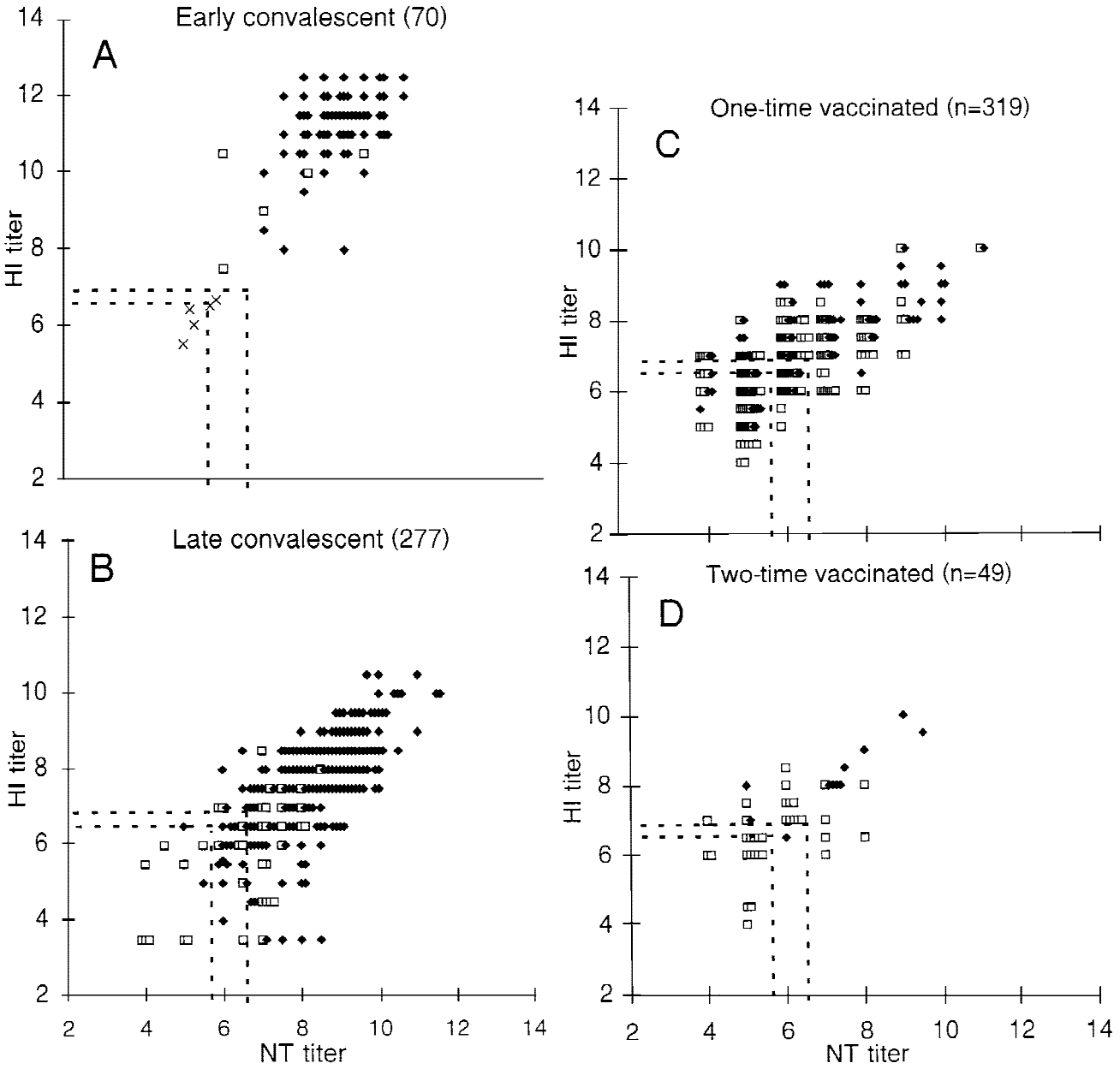

Fig. 2. Comparison of NT and HI titers of early **(A)** and late **(B)** measles convalescent donors and once **(C)** or twice **(D)** vaccinated high school children. Titers were measured as $\log_2$ dilutions (values ≤ 4 are negative). To avoid overlapping of symbols, identical values of HI were shifted by 0.1. Open and closed symbols correspond to individuals below or above the IgG threshold (see text). Dotted lines define NT and HI threshold titers for secondary immune response. The area between dotted lines corresponds to the undefined zone. In panel A the reference sera are shown as × (see Materials and Methods).

between subpopulations susceptible and resistant to secondary immune response (male/female for secondary immune response susceptibility and secondary immune response resistance: 0.21 and 0.29). In the secondary-immune-response-susceptible and -resistant groups of vaccinees, a similar percentage (8.6 and 7.8%) was vaccinated before the age of 15 months. With a single exception, all secondary-immune-response-competent persons were vaccinated before the third birthday, whereas in the resistant group 17.2% were vaccinated after this date. The latter may have had contact with wild-type MV before being vaccinated, which would explain their higher titers. Alternatively, vaccination at a later age induces higher titers.

DISCUSSION

It has been observed in isolated populations of measles vaccinees that these were serologically boosted in the absence of clinical measles in the population [Pedersen et al., 1989]. This was interpreted as an indication that MV could survive and circulate in fully protected vaccinated individuals. We speculate that in the absence of measles, protected subjects with low ti-

TABLE I. Levels of Significance (P by Student Unpaired t-Test) of HI and NT Titers, With Levels of MV-Specific IgG Between Early and Late Convalescent Donors and Vaccinated High School Children[a]

	Early convalescent	Late convalescent	Vaccinated (once)
HI (P)			
Early convalescent			
Late convalescent	$<10^{-60}$		
Vaccinated (once)	$<10^{-100}$	$<10^{-6}$	
Vaccinated (twice)	$<10^{-38}$	n.s.	n.s.
NT (P)			
Early convalescent			
Late convalescent	$<10^{-4}$		
Vaccinated (once)	$<10^{-42}$	$<10^{-57}$	
Vaccinated (twice)	$<10^{-24}$	$<10^{-21}$	n.s.
MV-IgG (P)			
Early convalescent			
Late convalescent	$<10^{-27}$		
Vaccinated (once)	$<10^{-29}$	n.s.	
Vaccinated (twice)	$<10^{-13}$	n.s.	n.s.

[a]Values are given as exponentials and correspond to the data of Figure 2. Mean ± S.D. are shown in Figure 1; n.s. denotes not significant.

ters, i.e., those prone to develop asymptomatic secondary immune response, would be the most likely group to support viral transmission. Until now, wild-type virus has not been isolated from individuals with asymptomatic secondary immune response, possibly because these are poorly defined. But direct case confirmation of unapparent secondary immune response is also complicated by the need of a documented measles (virus) contact as well as pre- and postreexposure serum samples. It is therefore difficult to obtain direct estimates of the frequency of persons susceptible to asymptomatic secondary immune response. We based our estimation on the prereexposure serology of household-exposed parents who developed asymptomatic secondary immune response with a concomitant increase in specific IgG, NT, and HI titers [Muller et al., 1996; Huiss et al., 1997].

Among 44 exposed parents, 9% developed secondary immune response [Muller et al., 1996]. In a population of 277 adults with natural immunity after measles infection, 3.2–3.9% exhibited the prereexposure serological characteristics of the secondary-immune-response-competent parents. Among vaccinated children, up to one-third seemed to be susceptible to develop secondary immune response upon contact with a measles patient. Christenson and Böttiger [1994] showed that specific titers can be boosted by revaccination, while boosting late convalescents was not possible, demonstrating that vaccinees are more likely to undergo vaccine-induced secondary immune response than late convalescents. In our study, secondary immune response susceptibility was not reduced by a second vaccination, although revaccination reduces susceptibility to measles [Hutchins et al., 1990; Robertson et al., 1992; Tulchinsky et al., 1993].

Secondary immune response may present with unspecific symptoms, mild or even typical measles (symptomatic secondary immune response) [Aaby et al., 1986; Edmonson et al., 1990], or it may remain clinically silent [Pedersen et al., 1989]. The above estimates include seronegative individuals susceptible to disease and those prone to develop symptomatic or asymptomatic secondary immune response.

TABLE II. Estimated Frequency of Secondary Immune Response Susceptibles Among Early and Late Convalescent Donors and Vaccinated High School Children[a]

	Secondary immune response susceptible	
Cohorts	Minimum[b]	Maximum[c]
Early convalescent	0	0
Late convalescent	3.2	3.9
Vaccinated (once)	22.2	33.2
Vaccinated (twice)	32.6	32.6

[a]Secondary immune response susceptibles are defined as those persons who, for each one of the three serological parameter (specific IgG, NT, HI), are below the threshold, Minimal and maximal estimates depend on whether (for each of the three parameters) the lowest threshold (excluding the undefined zone) or the highest threshold (including the undefined zone) is considered.
[b]Excluding the undefined zone of Figure 2.
[c]Including the undefined zone Figure 2.

In the first instance, among the vaccinated children, 3–4% were seronegative by ELISA (cf. Materials and Methods), but less than half of these were also negative for HI and NT. Such seronegative vaccinees correspond to primary vaccine failures and must not be counted as secondary immune response susceptibles. These values are in agreement with the primary vaccine failure rates of other studies [Miller et al., 1995]. In the late convalescent cohort, <1% is seronegative, i.e., susceptible to clinical measles.

Symptomatic secondary immune response is mainly seen as secondary vaccine failure and is only rarely found in measles convalescents [Aaby et al., 1986; Edmonson et al., 1990; Miller et al., 1995]. Most estimates of secondary vaccine failure range from 0 [Gustafson et al., 1987; Anders et al., 1996] to about 5% of either the measles patients or the vaccinees [Edmonson et al., 1990; Ozanne and d'Halewyn, 1992]. Measurements of

symptomatic secondary immune response susceptibility in a population largely depend on MV exposure and on the sampling method.

Even when primary and secondary vaccine failures are taken into account, the data still suggest that a large proportion of vaccinees is susceptible to asymptomatic secondary immune response. Susceptibility to unapparent secondary immune response may be 5 to 8 times higher than to symptomatic secondary immune response. In a fully vaccinated population asymptomatic secondary immune response was found to be as high as 66% [Pedersen et al., 1989].

The study describes a population with a high risk for secondary immune response, in which viral replication could potentially be studied after measles exposure. Moreover, serological follow-up of voluntary contacts between secondary immune response susceptibles and accidentally measles-exposed secondary immune response susceptibles could shed light on the potential infectivity of individuals undergoing secondary immune response.

As a result of sustained vaccination programs, measles immunity will wane in the general population and will fall to the level of vaccine-induced immunity. Moreover, in the elderly, vaccine-induced immunity wanes faster than natural immunity [Markowitz et al., 1990; Christenson and Böttiger, 1994]. If secondary immune response susceptibility plays a role in the epidemiology of measles, this role is likely to grow in fully vaccinated populations where secondary immune response could affect 30% or more. Vaccination strategies as well as eradication programs may have to consider the increasing number of secondary immune response susceptibility in highly vaccinated populations.

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